COS cells were transfected with the gene library and probed with anti bodies specific for a schizont antigen. Using an enzyme-based-detection system, scientists identified the COS cells containing the relevant plasmids. The recombinant plasmids were recovered from the COS cell and the gene encoding the schizont antigen was isolated This gene will now be used to characterize the antigen. The scientists particularly want to know if the antigen is recognized by CTLs as well as antibodies from immune cattle (see below).

Identification of antigens recognized by CTLs

As mentioned earlier, CTLs are believed to be important in protecting cattle against East Coast fever. A major objective of ILRAD's theileriosis research is to identify the parasite antigens recognized by CTLs obtained from cattle that are immune to East Coast fever; such antigens will form the basis of an effective vaccine against the disease.

One way to determine if a particular parasite antigen is recognized by CTLs is to transfect the gene encoding the antigen into a cell line expressing the appropriate bovine MHC class I molecule, such as the mouse fibroblasts transfected with bovine MHC class I molecules, as described above. These cells can then be used as target cells in a cytotoxicity assay to determine whether the gene encodes an antigen recognized by CTLs from an immune animal. In this way, it may be possible to identify from an array of candidate antigens those that could induce a protective CTL response. These antigens would form the basis of an effective vaccine against East Coast fever.

This article is based on reports of ILRAD studies of gene transfection written by Noel Murphy (trypanosomes) and Philip Toye (mammalian cells). Other ILRAD scientists involved in the mammalian cell transfection studies were Niall MacHugh, Ivan Morrison (now at the Compton Laboratory, UK), Jan Naessens and Alan Teale. Collaborators in this work include Piet Borst, of the Netherlands Cancer Institute (Amsterdam), and Hans Clevers and colleagues at the University Hospital, Utrecht, the Netherlands. The following article (page 5) is based on a report by former staff member Vinand Nantulya.

CORRECTION
The wording of a paragraph (page 2, last paragraph) in the recently published *ILRAD 1991 Highlights* is misleading. The paragraph should read as follows:

During the year, ILRAD scientists also identified a 24-kDa molecule present in infected bovine lymphocytes. The purified molecule was recognized *in vitro* by clones of helper T cells derived from immune cattle. The role of the 24-kDa antigen in inducing protective responses to *T. parva* is being investigated

New tools for better diagnosis of African trypanosomiasis

DIAGNOSIS is a critical element in the management of disease, both at the level of the individual animal when the decision to treat or not to treat has to be taken and for evaluating the success of disease control programs. The diagnostic tests used should be simple, rapid, specific and highly sensitive. They should also be able to differentiate between closely related parasite species if the disease syndromes they cause require different management approaches. Ideal tests should be suitable for field application and the cost should be within the means of the communities affected by the disease. ILRAD has developed simple tests for diagnosis of African trypanosomiasis that promise to fulfil many of these requirements.

Trypanosomiasis is characterized by severe anaemia, weight loss, reduced productivity, infertility and abortion, with death occurring in some animals during the acute phase of the disease. Animals that survive often remain infected with trypanosomes for several months or years and exhibit a low level of fluctuating parasitaemia. During this phase, the animals may manifest no overt clinical signs.

The standard laboratory method for confirmation of the diagnosis of African trypanosomiasis is to identify trypanosomes in tissues of the infected host, usually in peripheral blood. This method, however, is unsatisfactory, because a high proportion of infections are not detected. Alternative methods of diagnosis have therefore been developed, most of which are for the detection of antibody responses to the antigens of the infecting trypanosomes. Antibody detection tests, however, have several shortcomings: the antigens used are ill-defined, thus making standardization of the tests difficult with regard to sensitivity and specificity. Moreover, detection of antibody in serum does not necessarily reflect an existing infection, because antibodies may persist for several months following recovery.

Although undetectable in peripheral blood, trypanosomes can still be found sequestered in several other tissues of the infected host, such as the spleen, lymph-nodes, bone marrow and liver. Successive trypanosome variable antigen types in these populations are destroyed by the immune responses of the infected host as it attempts to eliminate the parasites. Several soluble antigens are thus released in the tissue fluids, including some that are trypanosome species-specific. Detection of these antigens thus provides direct evidence that an animal has a current infection. This is the rationale of the antigen-detection enzyme immunosorbent assays (antigen ELISAs) that have been developed for diagnosing African trypanosomiasis.

ILRAD scientists have developed three tests: one is specific for the parasite species *Trypanosoma vivax,* one for *T*. *congolense* and one for the *brucei* group of trypanosomes, which includes animal-infective *T*. *brucei* and *T*. *evansi* and human-infective *T*. *rhodesiense and T. gambiense*.

INITIAL EVALUATION of the tests for animal trypanosomiasis was conducted with staff from the Kenya Trypanosomiasis Research Institute (Muguga). Field sera were obtained from cattle in a trypanosomiasis-endemic area at Nguruman, Kenya. The three tests were able to detect trypanosome antigens in the sera of 121 (96.0%0) of 126 animals with parasitologically confirmed diagnosis. More importantly, the tests also detected antigens in 52.6% of animals in the same herd that had not been diagnosed as infected by parasite detection techniques, thus demonstrating the superior sensitivity of the antigen-ELISAs. Use of the latter tests enabled scientists also to detect mixed infections involving two or three trypanosome species. Furthermore, these assays proved highly specific: control sera obtained from cattle in a trypanosomiasis-free area of Kenya (at Kapiti) all tested negative using the three assays.

Applied to the diagnosis of *T. evansi* infections in camels and pigs, the test for Trypanozoon-specific antigens was positive in 92% of the animals with detectable parasitaemia. Once again, infections in a high proportion of animals undetected by parasitological techniques (55%) were detected using the antigen-ELISA. Control animals from a *T. evansi*-free area all tested negative with the latter.

FOLLOWING the initial successful field testing in Kenya, the tests were introduced for further evaluation in Ghana, Mali, Senegal, The Gambia, Tanzania, Zambia, Zimbabwe and Uganda. These field evaluation activities have been supported by the Government of the Netherlands in a collaborative project involving the Food and Agriculture Organization of the United Nations (FAO, Rome) and the International Atomic Energy Agency (IAEA, Vienna). The test specific for the *T*. *brucei* group has also been evaluated for its utility in diagnosing *T. evansi* infections in camels in Mali and Kenya and in diagnosing human trypanosomiasis in Côte d'Ivoire, Tanzania, Uganda, Zambia and Zaire. This evaluation was conducted under the auspices of the United Nations Development Programme/World Health Organization/World Bank Special Programme for Research and Training in Tropical Diseases (Geneva).

Results of these validation exercises were presented at an FAO/IAEA Research Coordination Meeting held in Côte d'Ivoire in May 1991. Participants of this meeting concluded that the two antigen-ELISAs that detect species-specific invariant trypanosome

antigens have been successfully introduced and established at 10 of the 11 institutes involved in the validation exercise. The participants reported that in detecting active trypanosome infections, the antigen-ELISAs demonstrated a sensitivity 4–5 times greater than that of the more commonly used buffy coat technique. It was concluded that the antigen-ELISAs clearly have potential use in the development of strategies for monitoring and controlling animal trypanosomiasis.

Clearance of antibody–VSG complexes by trypanosomes (Ph.D.Thesis)

CATTLE INFECTED with trypanosome parasites are known to produce antibodies against molecules known as VSGs that are located on the surface of trypanosomes. Host anti-bodies bound to parasite VSG mediate the killing of the parasites by other elements of the bovine immune system, such as macrophages and neutrophils. This is the main method by which the immune system clears trypanosomes from the body. It is also known, however, that trypanosomes can remove antibody bound to their VSG. Because this antibody removal has important implications for the control of the parasites, studies were undertaken to determine the trypanosome mechanisms responsible for cleaving VSG-antibody complexes.

The binding of antibodies that specifically recognize VSG molecules exposed on the surface of clones of *Trypanosoma brucei, Trypanosoma congolense* and *Trypanosoma vivax* was investigated using both immunofluorescence (light microscopy and a fluorescence-activated cell sorter) and electron microscopy. In addition, parasites of the *T. brucei S*427 clone 22, which were adapted to cultures containing no feeder cells, were incubated with specific F(ab)2 and Fab antibody fragments or with biotin.

After incubation at 0°C, antibodies, antibody fragments and biotin molecules were observed over the whole parasite surface; fluorescence appeared strongest at the parasite's flagellum. Upon warming to 37°C, surface-bound antibody and antibody fragments were cleared from the parasite surface. Even in the absence of antibody-mediated crosslinking of VSG (i.e., Fab), clearance occurred through the movement of surface-bound Fab-VSG complexes toward the flagellar pocket. Studies of permeabilized trypanosomes using electron microscopy and immunofluorescence showed that after being cleared from the cell surface, small amounts of antibody were located intracellularly between the nucleus and the flagellar pocket. However, when a cocktail of protease inhibitors was added to the culture medium, large amounts of antibody or antibody fragments could be detected within vacuoles situated between the nucleus and the flagellar pocket, suggesting that proteases are required to break down antibody–VSG complexes. Different antibodies were cleared at different rates. Antibodies with both a higher molecular mass and more than one antigen-binding site were generally cleared most rapidly.

Movement of antibody-VSG complexes was inhibited at temperatures below 4°C and by adding 2-deoxy-Dglucose in lieu of D-glucose to the culture medium. Movement was immediately and reversibly inhibited by increasing the NaCl concentration in the medium to 200 mM. Antibody clearance was also inhibited by protein synthesis inhibitors and protease inhibitors. The process was not inhibited by microfilament (cytochalasin B and D) or microtubule (nocodazole) disrupters, nor was it altered by an increase in medium viscosity.

IN SUMMARY, the results of these studies showed that antibody clearance in trypanosomes is a directional, energy-dependent process. It is not dependent on crosslinking of VSG and it is selective: only VSG bound to antibody is cleared.

Antibody clearance may have an important role to play in the process of antigenic switching in trypanosomes in which the parasites periodically remove one coat of VSG and replace it with another of a different antigenic type. An antibody response to a